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GUEST EDITORIAL

Moving from rats to cellular omics in regulatory toxicology: great challenge toward sustainability or "up-shit-creek without a paddle"?

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One of the amenities of today's life is that we enjoy an unprecedented level of consumer safety. The fact that we can go along our daily business without having to worry too much about major health risks is a tremendous success of regulatory toxicology. This comprises chemicals, foodstuffs, pesticides, biocides and consumer products such as commodities or cosmetics alike. Apart from a few exceptions, the underlying legal framework mostly relies on the results of live animal testing routinely performed according to harmonized guidelines of the OECD (http://www.oecd.org). The high level of scientific trust into this system is best reflected by the fact that data generated according to these guidelines are subject to mutual acceptance in all OECD member countries, thus avoiding an unnecessary repetition of animal tests while still providing producers and manufacturers with the required legal certainty.

However, with the advent of cellular screens, omics-based technologies and high-throughput assays, the established system has increasingly come under (scientific and public) scrutiny—less so for being ineffective but for aspirations to improve safety further and to address known shortfalls. This includes the issue of species specificity, the inadequacy to address mixtures or the effects of environmental background exposures as well as the need to evaluate potential endocrine disruptors and possible low-dose effects. Other aspects are testing efficiency and the ethics of animal testing (Hartung 2009; Kandárová and Letašiová 2011; Liebsch et al. 2011; National Research Council 2007; van der Jagt et al. 2004). In recent years, the push to exploit alternative testing for regulatory purposes has therefore led to large scale programs such as Tox21 (http://epa.gov/ncct/Tox21/), ToxCast (http://www.epa.gov/ncct/toxcast/), ExpoCast (http://www.epa.gov/ncct/expocast/), SEURAT (http://www.seurat-1.eu/) or the adverse outcome pathway (AOP) initiative of the OECD (OECD 2013). Yet, the question of how to use and to build up data from such alternative approaches into meaningful and effective regulation remains at the center of a heated debate.

In its core, this debate culminates mostly on the one question of how alternative safety testing can provide a sufficient or even increased level of protection—if at all (Adler et al. 2011; Andersen and Krewski 2010; Calabrese 2011; Hartung 2011; Judson et al. 2010; Kavlock et al. 2012; Keller et al. 2012; Tralau et al. 2012). While regulators tend to take a cautious stand in this matter, advocates of alternative testing continue to stress the benefits to be gained and point to tailored risk assessments and high-throughput testing. It helps to look at the roots of regulatory toxicology in order to appreciate the full complexity of this discussion. Toxicity testing arose from the pressing need to cope with the challenges of food and drug adulteration as well as the effects of industrial poisons (Oser 1987). The technical demands for such a testing system were the same as they are today: It had to be readily available and cost effective and come as close to human physiology as possible. Unsurprisingly, it was animals that sprang to mind. The more so because they already had a history of live hazard indicators as exemplified by the use of canaries and mice for the detection of carbon monoxide in coal mines since the eighteenth century. While humans are unarguably neither oversized birds nor rats, animal-based testing has fitted the bill ever since and mostly stood the test of time (Zbinden 1993).

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The big advantage of whole animal testing is the fact that it provides the most integrative results possible, with adverse effects being directly measurable and quantifiable on tissue, organ and organism level. In addition, in vivo testing accounts for plasticity, telling toxicologists to which concentration and to what extent a particular damage is reversible. It is this all-inclusive black-box principle that makes animal testing so powerful. At the same time, this strength is the biggest weakness as the respective endpoints usually do not provide any clues about the underlying molecular and cellular mechanisms (Fig. 1). Without this level of understanding, the transferability of results is restricted. This becomes apparent by the aforementioned systemic limitations such as species specificity but also causes problems for the regulatory evaluation of the increasing number of study results that do not comply with OECD guidelines (e.g., for bisphenol A or glyphosate).

This limited compatibility of in vivo-based histopathological endpoints to molecular or cellular readouts has to be overcome if the long-term vision of animal-free testing is to succeed. And for sure, the restricted "connectivity" between adverse in vivo findings and molecular endpoints need to be tackled in both kinds of directions, that is, "top-down" *and* "bottom-up" (i.e., histopathology upon killing *vs.* molecular indicators at single cell level, and vice versa; Fig. 1). Academic concepts such as the toxome (http://humantoxome.com/) address this issue by using the

tools of molecular biology, cell biology and systems biology to dissect cellular adversity (Bouhifd et al. 2014; Hartung and McBride 2011). Scientifically, this is unarguably the most consequent approach. However, although this is promising in the long term, it lacks immediate regulatory potential due to large knowledge gaps regarding molecular, cellular and tissue specific interactions. On the other side, programs such as ToxCast set their remit to the immediate use of alternative assays for screening and subsequent testing prioritization. By its completion in 2013, ToxCast had screened more than 2000 chemicals in more than 700 assays. The AOP initiative of the OECD on the other hand restricts itself to define single pathways such as "skin sensitization initiated by covalent binding to proteins" where there is sufficient molecular information to recapitulate adverse key events (OECD 2012a, b). A concomitant option is the use of physiology-based toxicokinetic (PBTK) modeling and organ-specific cellular assays in carefully selected pilot studies (Tralau et al. 2012).

However, regulatory application and acceptance require more than the demonstration of scientific feasibility. First of all, regulators are used to deal with readouts that can be translated into quantitative risk assessments, something that many of the current assays cannot yet provide. Regulators therefore often tend to see the immediate use of alternative testing primarily for hazard identification and biomarker identification. Also, one will have to account for the fact
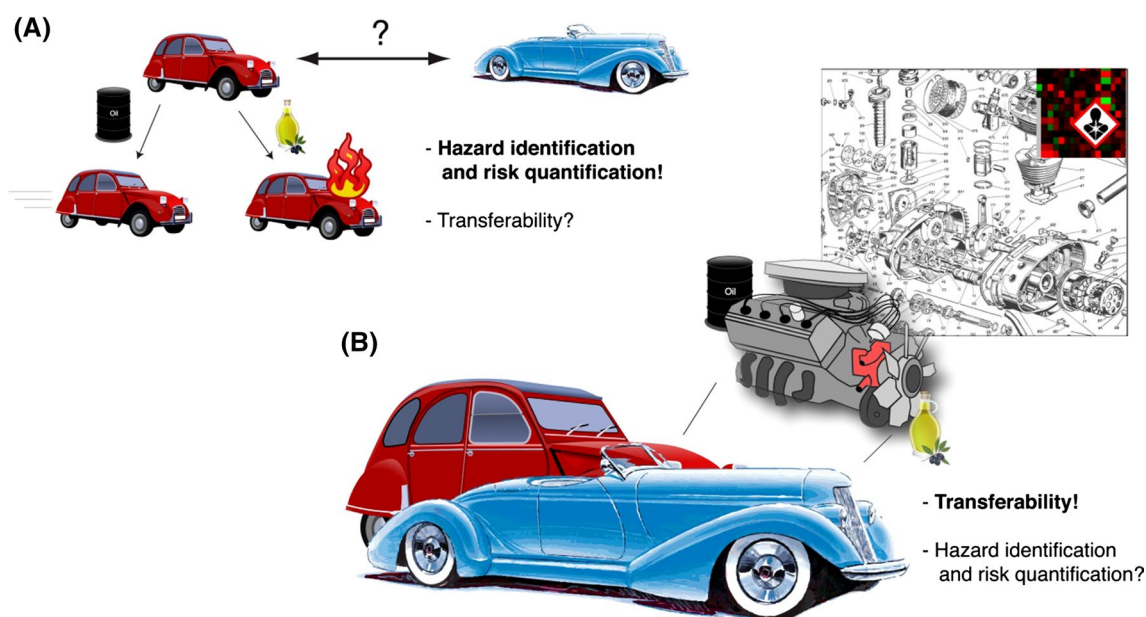

Fig. 1 Black-box testing versus dissectional analysis. **a** Systemic testing provides quantifiable results regarding the behavior of the whole system. However, because of the black-box principle, the results are of limited transferability. **b** Dissectional analysis, on the other hand, looks for effects on universal key components within

complex "organisms" that drive individual organs or "machineries" as integrative parts of the whole. Yet, without further comprehensive interpretation of the results originating from these "bits and pieces," any meaningful readout for the entire system and its behavior will remain unamenable

that cellular assays lack plasticity and are thus prone to over predictability. This leads to another unresolved issue, that is, assay validation. The multitude of assays and the pace of assay development make it unrealistic to subject them to validation as practiced currently (Judson et al. 2013). With many cellular test systems being based on human cells, it also has to be questioned if the current practice of defining animal tests as the "gold standard" really is the best way to go. The more so because these data are also used to develop and refine (Q)SAR models and in silico approaches. Moreover, apart from validation, assay acceptance requires reassurance that the results fit to the knowledge already gathered. It will therefore be important to see how the data of the current screening initiatives compare to conclusions drawn from existing in vivo data.

The discussion on alternative testing has long been perceived as being predominantly a scientific–academic one. However, it not only asks for a rethinking regarding key aspects of how regulatory toxicology is currently practiced but rattles at a complex legal system which is not designed and prepared to be changed quickly. Altogether, the task of implementing a more mechanistic approach for regulatory toxicology is a modern "Janus Gate", a waymark that once passed will soon refer many established practices to the past. With the direction already set, regulators are therefore called up on to participate and shape this process in order to move forward without compromising established and well-tested safety standards. The paper of Tralau et al. in this issue summarizes the current discussions at the German Federal Institute for Risk Assessment. Setting prominent aspects of alternative toxicity testing against the current *status quo*, it highlights major regulatory challenges and suggests some solutions.

References

Adler S, Basketter D, Creton S, Pelkonen O, van Benthem J, Zuang V, Andersen KE, Angers-Loustau A, Aptula A, Bal-Price A, Benfenati E, Bernauer U, Bessems J, Bois FY, Boobis A, Brandon E, Bremer S, Broschard T, Casati S, Coecke S, Corvi R, Cronin M, Daston G, Dekant W, Felter S, Grignard E, Gundert-Remy U, Heinonen T, Kimber I, Kleinjans J, Komulainen H, Kreiling R, Kreysa J, Leite SB, Loizou G, Maxwell G, Mazzatorta P, Munn S, Pfuhler S, Phrakonkham P, Piersma A, Poth A, Prieto P, Repetto G, Rogiers V, Schoeters G, Schwarz M, Serafimova R, Tahti H, Testai E, van Delft J, van Loveren H, Vinken M, Worth A, Zaldivar JM (2011) Alternative (non-animal) methods for cosmetics testing: current status and future prospects-2010. Arch Toxicol 85:367–485

Andersen ME, Krewski D (2010) The vision of toxicity testing in the 21st century: moving from discussion to action. Toxicol Sci 117(1):17–24

Bouhifd M, Hogberg HT, Kleensang A, Maertens A, Zhao L, Hartung T (2014) Mapping the human toxome by systems toxicology. Basic Clin Pharmacol Toxicol 115:24–31

Calabrese EJ (2011) Toxicology rewrites its history and rethinks its future: giving equal focus to both harmful and beneficial effects. Environ Toxicol Chem 30(12):2658–2673

Hartung T (2009) Toxicology for the twenty-first century. Nature 460(7252):208–212

Hartung T (2011) From alternative methods to a new toxicology. Eur J Pharm Biopharm 77(3):338–349

Hartung T, McBride M (2011) Food for thought… on mapping the human toxome. ALTEX 28(2):83–93

Judson RS, Houck KA, Kavlock RJ, Knudsen TB, Martin MT, Mortensen HM, Reif DM, Rotroff DM, Shah I, Richard AM, Dix DJ (2010) *In vitro* screening of environmental chemicals for targeted testing prioritization: the ToxCast project. Environ Health Perspect 118(4):485–492

Judson R, Kavlock R, Martin M, Reif D, Houck K, Knudsen T, Richard A, Tice RR, Whelan M, Xia M, Huang R, Austin C, Daston G, Hartung T, Fowle JR, Wooge W, Tong W, Dix D (2013) Perspectives on validation of high-throughput assays supporting 21st century toxicity testing. ALTEX 30(1):51–66

Kandárová H, Letašiová S (2011) Alternative methods in toxicology: pre-validated and validated methods. Interdiscip Toxicol 4(3):107–113

Kavlock R, Chandler K, Houck K, Hunter S, Judson R, Kleinstreuer N, Knudsen T, Martin M, Padilla S, Reif D, Richard A, Rotroff D, Sipes N, Dix D (2012) Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. Chem Res Toxicol 25(7):1287–1302

Keller DA, Juberg DR, Catlin N, Farland WH, Hess FG, Wolf DC, Doerrer NG (2012) Identification and characterization of adverse effects in 21st century toxicology. Toxicol Sci 126(2):291–297

Liebsch M, Grune B, Seiler A, Butzke D, Oelgeschläger M, Pirow R, Adler S, Riebeling C, Luch A (2011) Alternatives to animal testing: current status and future perspectives. Arch Toxicol 85(8):841–858

National Research Council (2007) Toxicity testing in the 21st century: A vision and a strategy. The National Academies Press, Washington, DC

OECD (2012a) The adverse outcome pathway for skin sensitisation initiated by covalent binding to proteins. Part 1: scientific evidence, series on testing and assessment 168, Paris

OECD (2012b) The adverse outcome pathway for skin sensitisation initiated by covalent binding to proteins. Part 2: use of the AOP to develop chemical categories and integrated assessment and testing approaches, series on testing and assessment 168, Paris

OECD (2013) Guidance document on developing and assessing adverse outcome pathways, series on testing and assessment GD 184, Paris

Oser BL (1987) Toxicology then and now. Regul Toxicol Pharmacol 7(4):427–443

Tralau T, Riebeling C, Pirow R, Oelgeschläger M, Seiler A, Liebsch M, Luch A (2012) Wind of change challenges toxicological regulators. Environ Health Perspect 120(11):1489–1494

van der Jagt K, Munn S, Tørsløv J, de Bruijn J (2004) Alternative approaches can reduce the use of test animals under REACH, Report EUR 21405 EN, European Commission Joint Research Centre

Zbinden G (1993) The concept of multispecies testing in industrial toxicology. Regul Toxicol Pharmacol 17(1):85–94